

REMARKS

Claims 1-51 are active in the present application. The claims are amended to remove multiple dependencies. No new matter is added. An action on the merits and allowance of the claims is solicited.

Respectfully submitted,

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IN THE CLAIMS

Please amend the claims as follows:

--3. (Amended) Method according to [either of claims 1 and 2] claim 1, characterized in that step c) for culturing said cells comprises a series of said cells in a culture medium containing the amino acid encoded by said target codon, each of said cultures of the series being prepared as far as obtaining the stationary growth phase and followed by washing of the cells obtained, the number of cultures of the series being sufficient to allow the selection of mutations which increase the suppression of said missense mutation of said mutated gene.

4. (Amended) Method according to [one of claims 1 to 3] claim 1, characterized in that the missense mutation is chosen from missense mutations which spontaneously reverse only at very low frequency, of the order of one organism from at least 10^{15} .

5. (Amended) Method according to [one of claims 1 to 4] claim 1, characterized in that the missense mutation transforms a target codon of a gene encoding a protein required for the growth of said cell, into a codon which, in comparison with the target codon, exhibits a change of at least two bases, preferably three bases.

6. (Amended) Method according to [one of claims 1 to 5] claim 1, characterized in that the target codon encodes an amino acid which has a small steric volume.

7. (Amended) Method according to [one of claims 1 to 6], claim 1, characterized in

that the target codon encodes an amphiphilic amino acid.

8. (Amended) Method according to [one of claims 1 to 7] claim 1, characterized in that the target codon encodes an amino acid which has a steric volume smaller than or substantially equal to the steric volume of the amino acid encoded by the missense mutation.

9. (Amended) Method according to [one of claims 5 to 8] claim 5, characterized in that the target codon encodes cysteine.

10. (Amended) Method according to [one of claims 5 to 9] claim 5, characterized in that the amino acid encoded by the missense mutation is valine or isoleucine.

11. (Amended) Method according to [one of claims 1 to 10] claim 1, characterized in that step a) for transforming said cells is carried out using a vector comprising a sequence of said gene encoding a protein required for the growth of said cells, including said missense mutation.

13. (Amended) Method for selecting cells capable of producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises steps a), where appropriate b), and c) of a method according to [one of claims 1 to 12] claim 1, and selecting the cells capable of growing in step c).

15. (Amended) Method for selecting cells according to [either of claims 13 and 14] claim 13, characterized in that the aminoacyl-tRNA synthetase which recognizes the amino acid encoded by said missense mutation of said selected cells is capable of charging onto one of its associated tRNAs an unconventional amino acid or an amino acid other than said amino acid encoded by said missense mutation.

18. (Amended) Cell obtained using a method according to [one of claims 1 to 17] claim 1.

20. (Amended) Cell according to claim 18 [or 19], characterized in that it is a

prokaryotic or eukaryotic cell.

22. (Amended) Cell according to [one of claims 18 to 21] claim 18, characterized in that it is chosen from the following cells deposited at the CNCM (Collection Nationale de Culture de Microorganismes [National Collection of Microorganism Cultures], Paris, France):

- a) *E. coli* strain deposited at the CNCM under the No. I-2025 on May 25, 1998,
- b) *E. coli* strain deposited at the CNCM under the No. I-2026 on May 25, 1998,
- c) *E. coli* strain deposited at the CNCM under the No. I-2027 on May 25, 1998,
- d) *E. coli* strain deposited at the CNCM under the No. I-2339 on October 26, 1999,
- e) *E. coli* strain deposited at the CNCM under the No. I-2340 on October 26, 1999,

and

- f) *E. coli* strain deposited at the CNCM under the No. I-2341 on October 26, 1999.

23. (Amended) Use of a method according to [one of claims 1 to 17] claim 1 for producing protein the amino acid sequence of which comprises at least one unconventional amino acid.

24. (Amended) Use of a cell according to [one of claims 18 to 22] claim 18 for producing protein the amino acid sequence of which comprises at least one unconventional amino acid.

25. (Amended) Process for producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises the following steps:

- a) where appropriate, selecting a cell by a method according to [one of claims 13 to 17] claim 13;

- b) culturing said cell selected in step a) [or a cell according to one of claims 18 to 22]

in a culture medium and under culture conditions which allow the growth of said cell; and

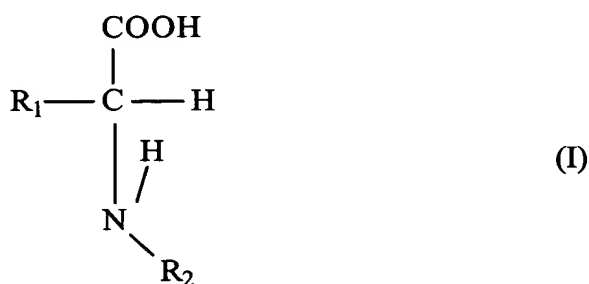
c) isolating said protein comprising at least one unconventional amino acid from the culture supernatant and/or from the cell pellet obtained in step b).

29. (Amended) Process according to [one of claims 25 to 28] claim 25, characterized in that said cell is auxotrophic for the amino acid encoded by said target codon.

30. (Amended) Process according to [one of claims 25 to 29] claim 25, characterized in that said cell comprises a homologous or heterologous gene of interest the coding sequence of which includes at least one target codon.

32. (Amended) Process according to claim 30 [or 31], characterized in that the biological activity of the protein encoded by said gene of interest is at least partially conserved after the incorporation of said unconventional amino acid at the level of the target codon of said gene of interest.

33. (Amended) Process according to [one of claims 25 to 32] claim 25, characterized in that the unconventional amino acid is chosen from unconventional amino acids of formula I of configuration L



in which:

R_1 or R_2 represents radicals containing a functional group capable of reacting

selectively.

35. (Amended) Process according to [one of claims 25 to 34] claim 25, for protein functionalization.

36. (Amended) Protein purification process, characterized in that it comprises the following steps:

a) incorporating into the amino acid sequence of said protein an unconventional amino acid containing a functional group capable of reacting selectively, using a process according to [one of claims 25 to 35] claim 25;

b) bringing the solution containing the protein obtained in step a) into contact with a support comprising a compound capable of reacting specifically with said functional group and of attaching specifically said protein; and

c) isolating said protein attached to the support.

37. (Amended) Process for attaching a protein to a chemical or biochemical compound, characterized in that it comprises the following steps:

a) incorporating into the amino acid sequence of said protein, by a process according to [one of claims 25 to 35] claim 25, an unconventional amino acid containing a functional group capable of reacting selectively;

b) bringing the protein obtained in step a) into contact with said chemical or biochemical compound comprising a group capable of reacting specifically with said functional group in a medium allowing the reaction.

41. (Amended) Process according to claim 39 [or 40], characterized in that the chemical or biochemical compound is chosen from compounds capable of modifying the biological activity of the attached protein.

42. (Amended) Process according to claim 39 [or 40], characterized in that the

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chemical or biochemical compound is chosen from compounds the biological activity of which can be modified by the attached protein.

43. (Amended) Process according to [one of claims 39 to 42] claim 39, characterized in that the chemical or biochemical compound is chosen from compounds comprising a protein, a polynucleotide, a fatty acid, a sugar or a natural or synthetic polymer.

44. (Amended) Protein obtained using a process according to [one of claims 25 to 36] claim 25.

46. (Amended) Protein complex obtained using a process according to [one of claims 39 to 43] claim 39.

47. (Amended) Use of a protein according to claim 44 [or 45, or of a protein complex according to claim 46], as a diagnostic reagent.

48. (Amended) Diagnostic process, characterized in that it uses a protein according to claim 44 [or 45, or a protein complex according to claim 46].

49. (Amended) Diagnostic pack, characterized in that it contains a protein according to claim 44 [or 45, or a protein complex according to claim 46].

50. (Amended) Use of a protein according to claim 44 [or 45, of a protein complex according to claim 46 or of a cell according to one of claims 18 to 22] for preparing a pharmaceutical or cosmetic composition.

51. (Amended) Pharmaceutical or cosmetic composition comprising a protein according to claim 44 [or 45, a protein complex according to claim 46 or a cell according to one of claims 18 to 22].--

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